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Potential Mechanisms Related to Salt Tolerance in Bean Plants

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Potential Mechanisms Related to Salt Tolerance in Bean Plants

By

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THESIS

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ABSTRACT

Phaseolus vulgaris (navy bean) and *P. acutifolius* (tepary bean) were grown hydroponically and stressed with -0.25 MPa NaCl for 9 days beginning 22 days after planting. Chloride and sodium distribution in leaves and roots as well as percent ion leakage as an indication of membrane integrity in leaves were determined. Chloride levels in NaCl treated plants were significantly higher than in controls. Chloride levels were not significantly different between plant parts or between plant species. Sodium distribution differed significantly between the roots of navy and tepary but not between the leaves of the two species. Navy root tissue contained twice as much sodium as the leaves, whereas, in tepary sodium concentrations were similar in leaves and roots. Significantly more sodium accumulated in NaCl treated plants than in controls. Percent ion leakage showed no notable trends among plant species or NaCl treatments, but a significant difference was observed between the two leaf ages.

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CHAPTER 1

INTRODUCTION

The development of agricultural societies allowed for centralization and urbanization of many cultures. Man's ability to raise crops en masse has been both beneficial and detrimental. A benefit was that large supplies of food could be grown in small areas and provide sustenance for large populations. However, agriculture was detrimental by causing considerable environmental stress. Land and water mismanagement have long aggravated environmental problems. One problem, salinity, was the focus of this study.

Farmers today are forced to farm some regions which should remain fallow due to urban sprawl and greater food demands. Today's society demands high yields from increasingly poorer soils where little water is available. Arid to semi-arid regions make up one-third of the world's land and one-half of this is composed of saline soils (Epstein, 1976). It is essential to develop crops which can withstand adverse environmental stresses, e.g., high salinity.

In order to produce crops which will thrive in a saline environment it is important to understand the physiological mechanisms involved in a plant's tolerance or susceptibility to the salt. Tepary bean and navy bean plants have obvious differences in salt tolerance. Tepary beans are salt tolerant while navy beans are salt sensitive leguminous plants. When stressed with sodium chloride, most salt tolerant leguminous plants exclude the ions from the leaves and sensitive plants accumulate it (Lauchli, 1984). Excess chloride causes leaf necrosis. Excess sodium generally results in reduced plant growth (Nieman, 1962). Tepary and navy bean plants may utilize different mechanisms in ion transport. If this theory is true, a difference in

sodium and chloride concentrations may be noted in specific plant parts such as leaf or root tissue. Salt stress in sensitive plants also may induce a loss of membrane integrity and allow ions to pass freely through the membrane.

The objectives in this study were two-fold: first, to determine whether tepary and navy beans partition sodium and chloride differently in different plant parts, and second, to determine how membrane integrity of leaf tissue is associated with tolerance to salt and accumulation of sodium and chloride.

CHAPTER 2

LITERATURE REVIEW

Sodium, chloride, sulfate, and bicarbonate salts are found in various concentrations in nature. Salt stress in nature is primarily due to sodium excesses, in particular sodium chloride (Levitt, 1980).

Salinity effects on plants are well documented (Aswathappa and Bachelard, 1986; Boursier et. al., 1987; Hajibagheri, Harvey and Flowers, 1987). These effects can be separated into three broad categories: water relationships, nutrition and energy balance (Pasternak, 1987). All plant processes interact; therefore, separation of these categories is not always clear.

Salts decrease osmotic potential of a solution. Some plants adjust to a saline environment by reducing internal osmotic potential (Bernstein, 1963; Bowman, 1988). Syvertsen, Lloyd, and Kriedemann, (1988) found that citrus leaf osmotic potential was reduced and by high salinity. A reduction in osmotic potential generally results in cell expansion due to increased water uptake. Some plants cannot adjust to salt excesses, generally a reduction of growth due to water stress (loss of turgor) results. In kenaf, increases in leaf area were more sensitive to salt stress than were leaf emergence rates and accumulation of dry matter (Curtis and Lauchli, 1986 and 1987). Growth reduction is a primary plant response to excess salinity (Neiman, 1962).

Direct toxicity and nutritional imbalance are two primary effects of excess salts on plants. Direct toxicity, resulting from excess accumulation of ions, and the effects of nutritional imbalance are not easily distinguished in plants. Sodium and chloride effects on plant

physiology and metabolism are well documented (Albert, 1975; Ayoub, 1975; Bowman, 1988; Syvertsen et. al., 1988). A study of halophytes in saline soils showed significant sodium and chloride accumulation within maturing leaf tissue and a decrease in potassium concentrations (Albert, 1975). Senna plants accumulated sodium and calcium in leaves while potassium and magnesium decreased with increased salinity (Ayoub, 1975). The primary mode of salt exclusion in these studies was shedding of mature leaves.

Plants utilize energy in the process of adaptation to excess salts. Ion transport across cell membranes can deplete energy supplies normally used for growth functions. In *Taxodium distichum* L., treated with several salt concentrations, photosynthetic rate decreased with increasing salt due to excess ion accumulation in leaf tissue (Pezeshki, DeLaune and Patrick, 1988). Decreased availability of photosynthate with increased salt concentrations was not responsible for growth reduction (Aslam et. al., 1986). It was proposed that growth limitations in *Atriplex amnicola* were due to deficiencies in organic solutes for osmotic regulation and not to high internal sodium chloride concentrations (Aslam et. al., 1986; Jeschke, Aslam and Greenway, 1986).

Ion concentration gradients in different age leaves or in leaves vs. roots have been well documented (Albert, 1975; Ayoub, 1975; Boursier, et. al. 1987; Hodson, Opik and Wainwright, 1985; Lauchli, 1984). A sodium and chloride concentration gradient, decreasing from old to young leaves, is a primary mode of salt regulation in many plants (Albert, 1975; Ayoub, 1975; Syvertsen et. al., 1988; Yeo and Flowers, 1982). Older leaves often are shed, reducing the ion concentration within the plant. Ion accumulation within two species of

Casuarina, differing in salt tolerance, were compared (Aswathappa and Bachelard, 1986). Sodium and chloride concentrations decreased from old to young needles in the tolerant species. No gradient was observed in the less salt tolerant species. Other plants control the movement of ions from the roots to the shoots. Sodium and chloride concentrations in salt tolerant varieties of maize were relatively high in roots and low in the shoots when compared to salt sensitive varieties (Hajibagheri et al., 1987). Orange trees maintained higher chloride concentration within the leaves compared to root tissue (Syvertsen et al., 1988). Modes of ion accumulation or elimination differed between salt sensitive and salt tolerant varieties of plants. Therefore, it is important to ascertain whether a contrast in ion accumulation modes exists between plants differing in salt tolerance.

Direct evidence of primary salt stress injury is not easily demonstrated. Increased membrane permeability to ions has been implied as a salt-induced injury (Levitt, 1980). That is, increased salt concentration can damage the membrane directly, affecting the influx and efflux of ions. Electrical conductivity can be used to measure a membrane's stability under salt stress, by comparing conductivity of solutions with salt stressed tissue to that of unstressed tissue. A high percent ion efflux in cells under salt stress can indicate primary membrane injury (Levitt, 1980).

Bean plants are affected by salinity in a number of ways. First trifoliate leaves of navy beans treated with sodium chloride were small compared to those of control plants (Wignarajah, Jennings and Handley, 1975). Treated leaves were thicker than control leaves due to increased cell volume within the spongy mesophyll. Dry matter and seed

yield decreased in mung beans with increasing sodium chloride concentrations (Salim and Pitman, 1988). At greater than 100 mM NaCl, leaves became necrotic and died. Seeds effectively excluded sodium and chloride and stems acted as a sink for the minerals. Similar results occurred in green soybean plants (Nukaya, Masus and Ishida, 1982). Ion selectivity in bean hypocotyl is due to the properties of the plasma membrane which are independent of the transport mechanism (Waisel, Neumann and Kuller, 1970). Bean hypocotyls had an apparent preference for the uptake of sodium over other monovalent ions. This uptake prevents sodium ions from moving from the roots across the hypocotyl into the shoots.

Beans have long been an important source of protein. The navy bean is important as a source of protein worldwide, especially in developing countries (Goertz, 1989). The climate in many of these regions generally is arid and salinity is often a problem. Navy beans are highly salt sensitive and generally are grown in temperate climates (Goertz, 1989). Tepary beans have been grown for centuries in arid regions and are salt tolerant. However, various economic and political changes lead to the decline in cultivation of the tepary bean. Teparies tend to have inconsistent and long cooking times compared with navy beans making them an impractical food source in many developing countries. The potential of tepary for cultivation in saline regions has caused renewed interest in its study.

Previous work on navy and tepary beans involved several parameters. Comparisons of salt tolerance at various stages of development were made. Adaptability to hot semi-arid climate, productivity vs. biomass, location of reproductive structures, temperature sensitivity, respiration, photosynthetic rates, and osmotic

potentials were compared (Goertz, 1989). In all, the tepary appeared better adapted to arid conditions than navy beans. The differences in these beans make them a good choice for comparisons in saline studies.

CHAPTER 3

MATERIALS AND METHODS

Plant Growth

White tepary bean (*Phaseolus acutifolius* Gray var. *latifolius* PI 440790 produced in 1988 in Arizona) and navy bean (*Phaseolus vulgaris* L. 'Sanilac' from Rogers Seed Co., Twin Falls, Idaho) were used in this study. Tepary seeds were untreated and navy seeds were treated with a dust of Captan, Thiram, and Lorsban. Seeds were planted in a styrofoam container with 70 2.5 cm X 5.1 cm holes. Thirty-five seeds were planted for each variety. A 1:1:1 (v/v/v) mixture of vermiculite:perlite:peat was used as support medium for the seeds. Seeds were watered daily from below with 600 ml tap water. Seedlings were watered, several days after emergence of plants with Peter's 20:20:20 soluble fertilizer at a rate of 1 tsp/l (600 ml/day per container).

Plants were grown in a growth chamber with average temperature of 25.2 $\pm$ 3.3°C as measured daily with a digital thermocouple thermometer (Wescor) throughout the experiment. Relative humidity was 69 $\pm$ 11% as measured weekly with an aspirated thermocouple psychrometer (handmade). The photoperiod was 16 hours of light and 8 hours of darkness. Light intensity as measured with a LI-COR light meter (model LI-185A) with a quantum sensor was 310 $\pm$ 130 micromoles m^{-2} sec^{-1} .

Plants were transferred to the hydroponic system 11 days after seeding, and were chosen on the basis of uniformity of size. Tepary plants had two trifoliate leaves and navy plants had at least one trifoliate leaf, with a second just expanding, at the time of the transfer. All plants had their cotyledonary leaves intact at this point. Roots were rinsed in deionized water to remove support medium

before entry into hydroponic system. Plants were entrained to wind around string hung from the top of the growth chamber. This string kept interplant twining to a minimum.

Hydroponic System

Plastic cylindrical tubs (2.2 liters-14 cm diameter X 19 cm tall) were covered with aluminum foil to deter algal growth. Tubs were filled with nutrient solution (see appendix A), and the tops were fitted with 1 inch thick polystyrene boards, with holes to accommodate the plant and aeration hose. Plants were supported with foam plugs covered with plastic wrap, to decrease contact of salts with plant stems.

Tubs were aerated with electromagnetic linear motion compressors (Apollo 5 model AM-5). Two pumps were used with each pump aerating 8 tubs. Standard airline tubing (4 mm diameter inside), gang valves, and aeration stones (1.3 cm. dia. x 2.5 cm long) for aquaria were used to connect the pump to each tub. Aeration stones were inspected regularly to maintain uniform air supply to each tub. Water levels were observed daily. Deionized water was added to maintain original water level.

Salt Treatments

At the five to six leaf stage (22 days after planting-DAP), hydroponic solutions were changed so that half the tubs contained just nutrient solution and the other half contained nutrient solution plus NaCl. The NaCl was added in a single dose. The control was 0.0 g NaCl/l and the salt treatment was 2.0 g/l NaCl(-0.25 MPa). The treatments were 2 NaCl levels X 2 species X 4 replications with 16

tubs which were completely randomized. The pH was 6.0 in both the new and original solutions. Each tub contained a single plant.

Sampling and Analyses

Membrane integrity of leaf tissue was determined by electrical conductivity (EC) analysis. The uppermost leaf was tagged with a string and label placed at the node 22 DAP. Two leaves were removed from each plant 30 DAP, one from above and one from below the marked leaf to represent leaves already fully expanded and those not yet expanded at 22 DAP. Leaves removed were separated by at least three leaves. The leaves were rinsed with deionized water and dried with a paper towel. Tissue for ion leakage analysis was collected by punching 12-7mm discs (4/leaflet) from each removed leaf avoiding major veins. Discs were placed in 25 ml of deionized water in individual tubes and tubes were covered and allowed to set for 12 hours to equilibrate (based on previous analysis, see appendix D). Once equilibrated conductivity measurements were made in uMHOS using an Altex conductivity bridge (model RC-16C) with Beckman conductivity cell (G01). The meter was set at 1 kHZ and the multiplier was adjusted as needed. Air temperature at the time of analysis was 22.6°C. The tubes were capped with aluminum foil, autoclaved at 15 psi 212°C for 15 minutes and cooled to room temperature before taking final readings. Room temperature was 22.3°C during the final reading. Percent ion leakage was determined (initial conductivity/ final conductivity * 100).

Plants were harvested 31 DAP for sodium and chloride analysis. Three leaves were sampled from each plant. Leaves were removed in an alternating pattern. The marked leaf (leaf # 5 or 6) was removed along

with a leaf above and below it alternating leaves (i.e. leaves no. 2, 5 [marked leaf], and 8 counting from the oldest leaves upward). Leaves always were taken from the same stem. Leaves were rinsed in deionized water and blotted dry with paper towel. Each leaflet on each leaf was cut in half lengthwise and the 3 leaflet halves (from same leaf) were combined in a beaker labeled for sodium or chloride analysis. Wet weight was determined for each sample. The samples were dried at 75°C for several days and placed in desiccation chambers with Drierite to cool. Dry weight was determined for each sample.

Root samples were taken from the lower portion of the root for sodium and chloride analysis. This tissue contained actively growing root tips as well as some mature tissue. Root tissue was rinsed first in deionized water and blotted dry. The roots were excised from the plant and half of the tissue was cut off crosswise. The tissue was divided in half longitudinally and placed in beakers labeled for sodium or chloride analysis. Dry weight was obtained as for leaf tissue, and sodium and chloride concentrations were determined.

For sodium analysis, the leaf or root tissue was placed in a 5 ml beaker, dry ashed and sodium determined by flame photometry (personal communication, R. Darding). Plant tissue was dry ashed at 500°C for about three hours, until no organic matter remained (no black color should remain in tissue). Once cooled to room temperature, the leaf or root material was dissolved in 5 ml of 15 mM LiCl. The flame photometer was set for Na analysis. A standard curve was prepared using 0 ppm, 25 ppm, and 50 ppm NaCl in 15 mM LiCl (see appendix B). The samples were analyzed for sodium content.

Chloride concentration was determined by potentiometric titration of chloride with $AgNO_3$ (LaCroix, Keeney and Walsh, 1970). The

remaining dry tissue (leaf and root) was shaken in 125 ml flasks with 0.1 N HNO_3 (50 ml 0.1 N HNO_3/sample) for 15 minutes. The mixtures were stirred rapidly while titrating with 0.0282 N $AgNO_3$:0.1 N HNO_3. A chloride ion-selective electrode (Corning model 476126) with Corning double junction reference electrode (model 476067) and a pH meter (Corning model 10) were used to measure chloride. The meter was set on the millivolt (mv) scale. The titrant was added in small increments and voltage was determined after each addition. The change in potential (E) per unit volume (V) of titrant (change E/change V; volts/ml) was plotted against volume of titrant (on semi-log paper) to determine the end point. The end point was indicated by the peak on the graph. Percent chloride per gram dry weight was calculated (see appendix C).

Statistical Analyses

Sodium concentrations, chloride concentrations and percent ion leakage were analyzed using analysis of variance with a completely randomized design. Differences between sodium, chloride and percent ion leakage means for species, plant parts and salt treatment were compared with Duncan's multiple range test at $P = 0.05$. Correlation coefficients between sodium, chloride and ion leakage were calculated.

CHAPTER 4

RESULTS

Tepary plants generally were more branched and produced more foliage than the navy plants, in both control and salt treatments. Navy plants grew compactly and appeared to have thicker leaves, particularly on salt stressed individuals. Thickening might be due to an increase in cell production in the spongy mesophyll (Wignarajah et. al., 1975). Yellowing was observed on lower leaves of some plants, and some of these leaves senesced. This response may be a salt regulation mechanism in some halophytes, as well as in some less salt tolerant plants (Albert, 1975; Ayoub, 1975; Cheeseman, 1988).

Three way analysis of variance on sodium concentrations revealed significant effects from salt and plant part but not from plant species (Appendix E; table E1). Analysis revealed a significant interaction between plant part and plant species with respect to sodium accumulation but other interactions were not significant (Appendix E; table E1). Due to significant interactions, data on each of the plant parts and each of the plant species were separated and two way analyses of variance were run independently. No significant interactions occurred in two way analyses of variance (Appendix E; tables E2-E7). When plant parts and bean species were lumped, mean sodium concentrations in salt treated plants were about 2.5 times higher than in control plants (Table 1). The analyses revealed a difference between sodium accumulation in navy and tepary plants. Differences between the species was in the way sodium was allocated within the plants. Mean sodium concentration was not significantly different among plant part, although roots and young leaves tended to have

Table 1. Sodium concentrations (% dry weight) in tissues[Z] of hydroponically grown bean plants[Y] treated with -0.25 MPa NaCl for 9 days beginning 22 days after planting (DAP).

NaCl Concentration (MPa)	Sodium Concentration (% dry weight)
-0.25 MPa	0.0034 a[X]
0.00 MPa	0.0014 b

[Z] values for all leaf stages and roots were combined because plant part did not interact with salt in 3 way ANOVA.
[Y] values for navy and tepary were combined because plant species did not interact with salt in 3 way ANOVA.
[X] mean separation within columns based on Duncan's multiple range at P=0.05.

higher sodium than middle and older leaves (Table 2). In navy plants sodium accumulation was quite different than in tepary. Mean percent sodium was similar at all leaf ages, but root tissue contained significantly more sodium than leaves (Table 2). A comparison of tepary and navy plants for each plant part separately showed similar sodium accumulation in both species for young, middle and old leaves, but in root tissue, navy contained significantly more sodium than tepary plants (Table 3).

Three way analysis of variance on chloride concentrations revealed no significance for plant part, plant species or interactions between plant part, plant species and/or salt (Appendix E, table E8). Since no significant interactions occurred, means were lumped to compare each parameter (Table 4). Salt treated plants contained more chloride than control plants (Table 4). All plant parts had similar chloride concentrations in their tissues, although a tendency for higher chloride in younger leaves was observed. Both tepary and navy accumulated similar concentrations of chloride.

Three way analysis of variance for percent leakage revealed no significant effect due to species, salt or interactions between plant species, plant part and/or salt (Appendix E, table E9). Salt treated plants showed slightly more leakage than controls, but not significantly so (Table 5). Analysis revealed both species had similar leakage (Table 5). The most profound contrast with regard to ion leakage was seen between plant parts. Percent ion leakage in young leaves was about two times that in older leaves (Table 5).

Sodium and chloride were significantly correlated when all treatments were considered (Table 6). Within control and salt treatments, sodium and chloride moved independently of each other.

Table 2. Sodium concentration (% dry weight) in leaves and roots of hydroponically grown bean plants with both control and salt treated values combined[z].

Plant Part	Bean Species	
	Tepary	Navy
Roots	0.0027 a[y]	0.0047 a
Old leaves	0.0017 a	0.0022 b
Middle leaves	0.0014 a	0.0016 b
Young leaves	0.0029 a	0.0020 b

[z] values for control and salt treated plants were combined because salt did not interact with plant part in 2 way ANOVA.
[y] mean separation within columns based on Duncan's multiple range at P=0.05.

Table 3. Sodium concentrations (% dry weight) in tissues of hydroponically grown tepary and navy plants with both control and salt treated values combined[Z].

Plant Species	Plant Part			
		Leaves		
	Roots	Old	Middle	Young
Tepary	0.0027 a[Y]	0.0017 a	0.0014 a	0.0029 a
Navy	0.0047 b	0.0022 a	0.0016 a	0.0020 a

[Z] values for control and salt treated plants were combined because salt did not interact with plant part in 2 way ANOVA.
[Y] mean separation within columns based on Duncan's multiple range at P=0.05.

Table 4. Chloride concentrations (% dry weight) in leaves and roots of hydroponically grown tepary and navy plants treated with -0.25 MPa NaCl for 9 days beginning 22 DAP.

Plant Part	Chloride concentration (% dry weight)
Root	0.99 a[z]
Old Leaf	0.96 a
Middle Leaf	1.10 a
Young Leaf	1.21 a
Bean Species	
Tepary	1.09 a
Navy	1.04 a
NaCl Concentration (MPa)	
-0.25 MPa	2.06 a
0.00 MPa	0.07 b

[z] mean separation within columns based on Duncan's multiple range at P=0.05.

Table 5. Percent leakage[z] in leaves of hydroponically grown tepary and navy plants treated with -0.25 MPa NaCl for 9 days beginning 22 DAP.

NaCl Concentration (MPa)	Percent Ion Leakage
-0.25 MPa	37.02 a
0.00 MPa	28.48 a
Plant Part	
Old Leaf	22.84 a[y]
Young Leaf	42.66 b
Bean Species	
Tepary	30.31 a
Navy	35.19 a

[z] each sample = 12-7mm leaf discs/25 ml deionized water. Percent leakage = initial electrical conductivity (E.C.) divided by final E.C. x 100.
[y] mean separation within each factor based on Duncan's multiple range at P=0.05.

Sodium and chloride were correlated in tepary but not in navy. The correlation coefficients differ only slightly with tepary at 0.38 and navy at 0.34, suggesting that more repetitions might increase the correlation between sodium and chloride for navy. Of particular interest is the strong positive correlation between sodium and chloride within middle and old leaves, while in young leaves and roots no such correlation was observed. Neither sodium nor chloride was significantly correlated to ion leakage in any treatment groups (Tables 7 and 8).

Table 6. Correlation coefficients between sodium and chloride in different treatment groups.

all treatments	0.35**[z]
all controls	-0.27ns
all salts	-0.16ns
all tepary	0.38*
all navy	0.34ns
all young leaves	0.27ns
all middle leaves	0.83***
all old leaves	0.80***
all roots	0.14ns

[z] ns,*,**,*** represents nonsignificant or significant at P = 0.05, 0.01 or 0.001, respectively.

Table 7. Correlation coefficients between sodium and ion leakage in different treatment groups.

all treatments	0.21ns[z]
all controls	0.03ns
all salts	0.09ns
all tepary	0.14ns
all navy	0.28ns
all young leaves	0.25ns
all old leaves	0.04ns

[z] ns=nonsignificant

Table 8. Correlation coefficients between chloride and ion leakage in different treatment groups.

all treatments	0.25ns[z]
all controls	-0.22ns
all salts	0.15ns
all tepary	0.26ns
all navy	0.28ns
all young leaves	0.22ns
all old leaves	0.24ns

[z] ns=nonsignificant

CHAPTER 5

DISCUSSION

Tepary and navy beans differ phenotypically. Goertz (1989) documented differences between tepary and navy for several parameters regarding salt tolerance. Tepary plants are salt tolerant relative to navy beans. Although direct growth measurements were not taken in the present study, some distinct differences were observed between tepary and navy plants. Tepary are vining and navy are bush-type plants by nature. Tepary plants were highly branched and appeared to produce comparable growth in both control and salt treatments. Navy plants seemed to branch less and to produce less foliage than tepary. Once stressed with salt, growth of navy plants slowed dramatically. Stressed navy plants produced two to three less leaves per branch than controls during the same period, supporting previous work on the salt sensitivity of navy bean plants.

Comparisons of sodium and chloride concentrations as well as membrane integrity of the two species, various plant parts and at two salt treatments helped to define better the differences between navy and tepary physiologically. The most outstanding difference observed between the two plant species was the location ofsodium accumulation within the plant. Navy plants maintained high root sodium levels compared to tepary. In navy plants, sodium concentrations in leaf tissue were about half that found in roots, and about equal in leaves. This observation contrasts with tepary which had no significant differences for sodium in different plant parts. These findings are in contrast to the findings of Hajibagheri et al. (1987), which showed salt tolerant varieties of maize had higher sodium and chloride

concentrations in the roots compared to the shoot. Differences in salt accumulation may be attributed simply to the differences between distribution of salts in monocot and dicot plants. Navy plants are quite salt sensitive, and it is possible these plants increase salt concentrations within the roots as an osmotic adjustment mechanism. Since tepary plants are salt tolerant, another mechanism may have evolved that utilizes other substances for osmotic adjustment. For example, soluble photosynthates will lower osmotic potential and enable the plant to continue growing.

Differences between salt treatments were quite pronounced. Control plants had substantially less sodium and chloride on a dry weight basis than salt treated plants. A comparison of control and salt treated plants did not reveal a significant difference in ion leakage. This lack of significant difference for ion leakage may indicate that salt has little effect on membrane integrity in bean species tested.

The mechanisms which these plants utilize to maintain growth under salt stress are uncertain, so the present study tested several hypotheses. Hypothesis one, suggests that time of exposure to stress is related directly to the amount of sodium and/or chloride located in the various plant parts. Since the youngest leaves were not present at the initiation of salt stress, and middle and old leaves were, less sodium and chloride would be expected in youngest leaf tissue. Results did not confirm this hypothesis. Although differences were not significant, tepary tended, if anything, to accumulate more sodium in young leaves and root tissue. Navy plants accumulated sodium equally from young to old leaves. Overall, chloride accumulation from young to old leaves was not significantly different. However, a trend was

observed with chloride concentrations decreasing from young to old leaves. These results indicate the length of time a leaf is exposed to salt stress does not reflect sodium or chloride content. Therefore, it is possible an energy dependent mechanism is utilzed which, moves the ions through the plant.

Hypothesis two, suggests a mechanism by which sodium and chloride are partitioned into older leaves or other plant parts, eliminating excess salts from the majority of the plant. Numerous studies show halophytes partition sodium and chloride into older leaves which are eventually shed (Albert, 1975; Ayoub, 1975; Yeo and Flowers, 1982). Neither tepary nor navy partitioned sodium or chloride in this manner. LaHaye and Epstein (1969) demonstrated that some glycophytes are capable of excluding sodium from the shoots to avoid salt injury with the plasmalemma of the root absorbing cells being the primary ion exclusion site. Navy plants maintained high root sodium and low leaf sodium levels, indicating that sodium was not prevented from entering the roots but was prevented from entering the stem of the plant Although a difference between tepary and navy with regard to sodium partitioning was obvious, the mechanisms each species utilizes are not yet clear.

Hypothesis three, suggests membrane integrity is linked to salt tolerance by allowing or preventing uptake of sodium and chloride. In this study salt tolerance had little effect on a plant's ability to regulate ion flow across membranes in plants stressed with salt. Navy and tepary plants were not significantly different with respect to ion leakage. Still, an interesting observation was made regarding ion leakage and plant parts. Young leaf tissue, which tended to have

higher sodium and chloride concentrations, also had a significantly higher ion leakage than did older leaf tissue. A connection may exist between salt concentrations in leaf tissue and ion leakage, although further analysis is necessary.

A few changes might have benefited this study. Several trends were observed regarding sodium and chloride accumulation within tepary and navy plants. The use of more repetitions or a longer stress exposure period might verify the significance of such trends. It would have been helpful to measure the electrical conductivity of the hydroponic system and determine whether any changes in osmotic pressure occurred during the stress stage. Any changes may have an effect on how a plant accumulates salts. Placing the plants in blocks as opposed to complete randomization would make the study a little tighter, eliminating possible effects that differences in temperature or lighting might have on the results.

In future studies, analysis of root membrane integrity at the tips as well as near the shoot could further enhance data on differences in sodium accumulation between navy and tepary. Analysis of sodium at the junction of stem and root and in the root tips could be useful, as well. This information would better isolate sodium and chloride movement within navy and tepary plants. The addition of a hybrid between navy and tepary would also be of interest in the future to determine which characteristics are transferred from tepary and navy. Field studies in saline soils would add even a further dimension to the study, and take into account other factors not included when doing growth chamber and hydroponic studies.

APPENDIX A

NUTRIENT SOLUTIONS

Nutrient solution for hydroponic system.

Flask 1

$Ca(NO_3)_2 \cdot 4H_2O$	268.8 g
Fe330	11.3 g

Add distilled water to make 1 liter. Add to hydroponic tubs at rate of:

1.37 ml nutrient solution/ 2.2 liter water on DAY 1

1.37 ml " " " " DAY 22

Flask 2

KNO_3	90.1	g
$MgSO_4$	112.4	g
KH_2PO_4	60.8	g
H_3BO_3	0.64	g
$MnCl_2 \cdot 4H_2O$	0.50	g
$ZnSO_4 \cdot 2H_2O$	0.27	g
$CuCl_2 \cdot 2H_2O$	0.056	g
MoO_3	0.010	g

Add distilled water to make 1 liter. Add to hydroponic tubs at rate of:

13.74 ml nutrient solution/ 2.2 liter water on DAY 1

13.74 ml " " " " DAY 22

(From Goertz, 1989)

APPENDIX B

Sodium Analysis

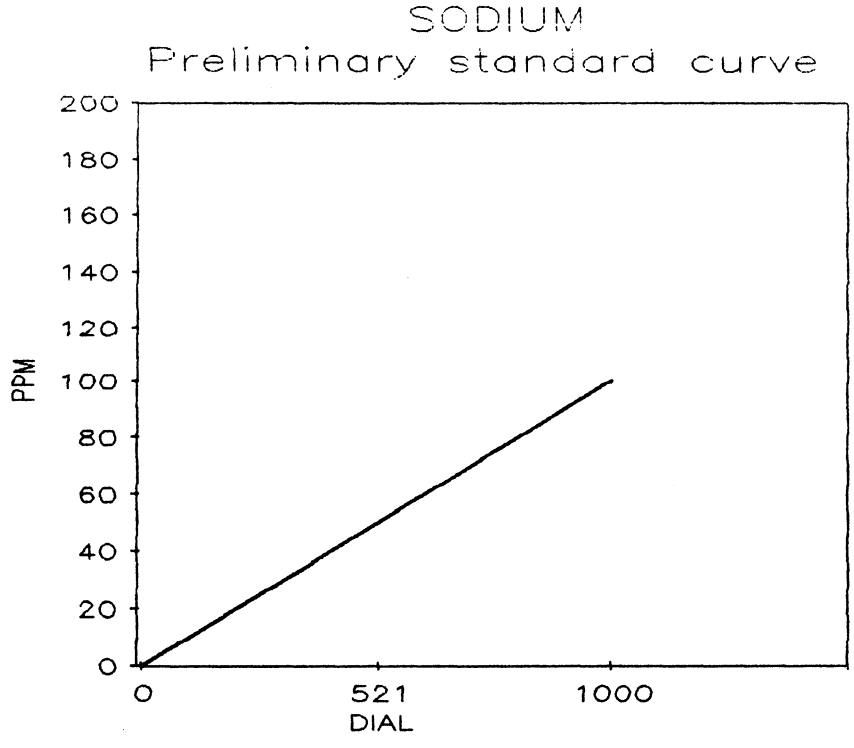
Preliminary sodium analysis:

Standard curve:

PPM SODIUM	DIAL READING
0	0
50	521
100	1000

Regression:

X variable	Y variable	Corr.	Slope (m)	Y int. (b)
DIAL	PPM	1.00	0.10	-0.67



Sample problem:

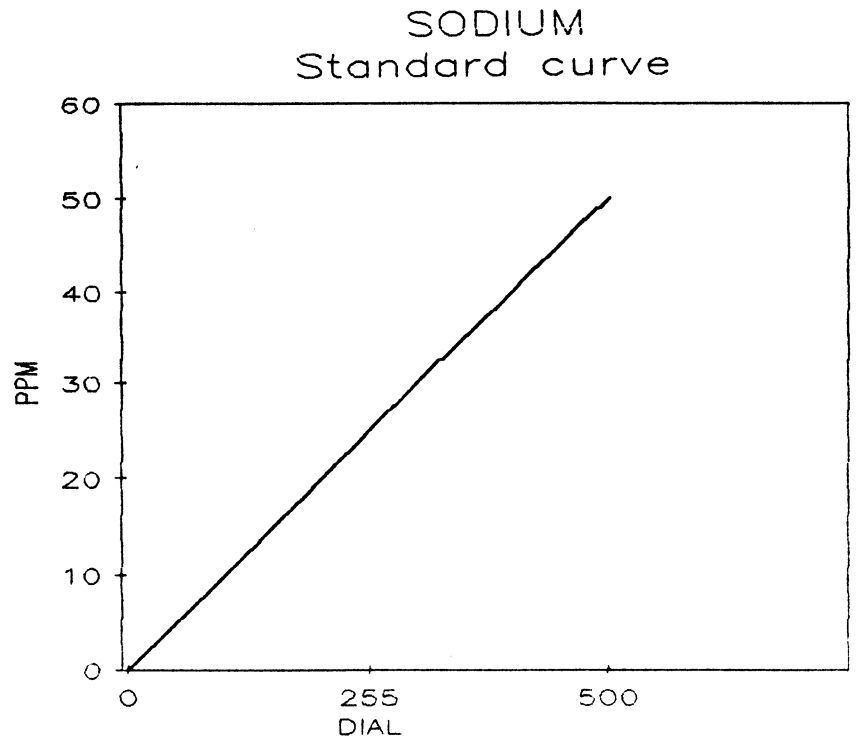
Y (ppm-Na)= m * X (dial) + b
PPM Na = 0.10 * DIAL + (-0.67)
PPM Na = 0.10 * 51 + (-0.67)
PPM Na = 4.43

Sodium analysis:
Sodium standard curve:

PPM SODIUM	DIAL READING
0	0
25	255
50	500

Regression:

X variable	Y variable	Corr	Slope (m)	Y int. (b)
DIAL	PPM	1.00	0.10	-0.16



CALCULATION-PPM SODIUM:

PPM SODIUM = 0.10 * DIAL + (-0.16)
PPM SODIUM = 0.10 * 166 + (-0.16)
PPM SODIUM = 16.4

CALCULATION-PERCENT SODIUM:

$$\frac{\text{parts sodium}}{1{,}000{,}000 \text{ parts tissue}} = \frac{\text{parts Na}}{100}$$

$$\frac{16.4 \text{ parts Na}}{1{,}000{,}000 \text{ parts tissue}} = \frac{\text{parts Na}}{100}$$

PERCENT SODIUM = 0.0016

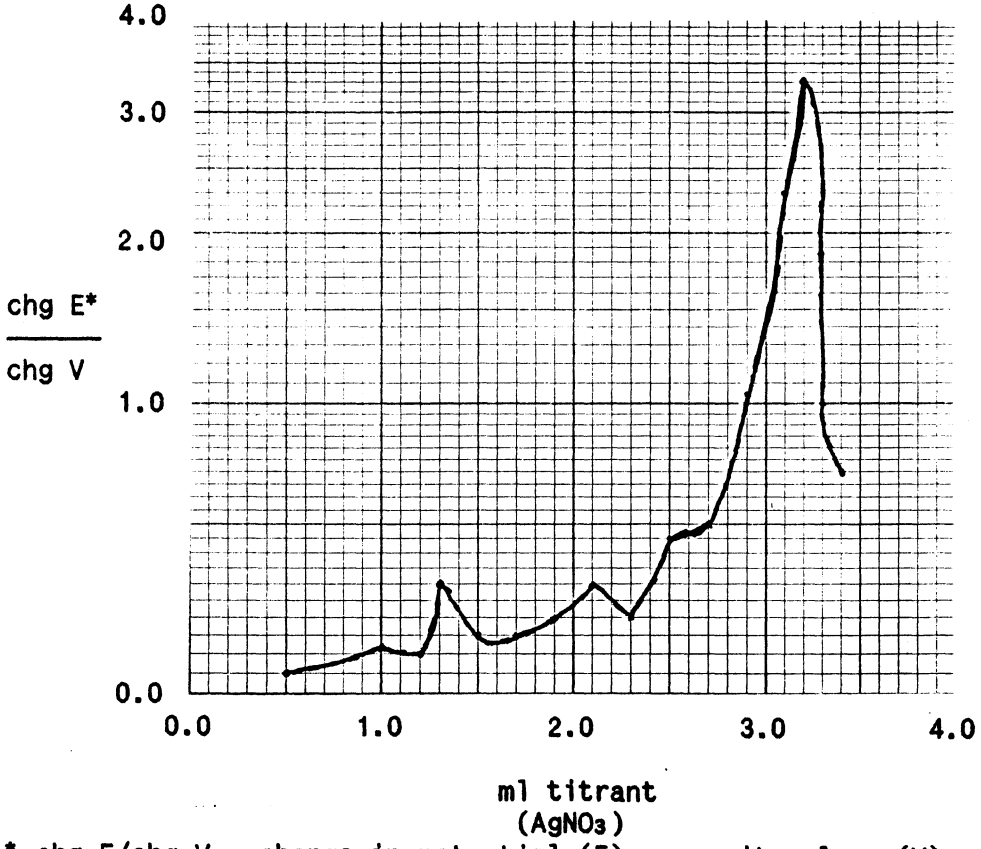
Appendix C

Chloride Analysis

Preliminary chloride analysis (on cantaloupe tissues with known chloride concentrations): Since this technique was new to the analyst, tissue previously analyzed for chloride was obtained (from Dr. Janice Coons) and values compared. Differences obtained might be attributed to differences in chloride analysis techniques or distribution of chloride in samples. However, our values were within range of previous values, so we felt our techniques were appropriate.

		PERCENT CHLORIDE	
plant #	leaf age	Previous values	our values
1	old	3.05	2.30
1	middle	2.70	2.60
1	young	3.77	2.90
2	old	2.33	2.10
2	middle	3.05	2.70
2	young	3.54	2.50

Chloride Titration Curve



* chg E/chg V = change in potential (E) per unit volume (V)

CALCULATION-PERCENT CHLORIDE:

$$\#\ ml\ AgNO_3 \left(\frac{1\ liter\ AgNO_3}{1000\ ml\ AgNO_3}\right)\left(\frac{0.0282\ moles\ Ag}{1\ liter\ AgNO_3}\right)\left(\frac{1\ mole\ Cl^-}{1\ mole\ Ag^+}\right) = \text{moles } Cl^-$$

$$\text{moles } Cl^- \left(\frac{35.45\ g\ Cl^-}{1\ mole\ Cl}\right)\left(\frac{}{g\ of\ plant\ tissue}\right) X\ 100 = \%\ Cl^-$$

Sample calculation:

$$3.20\ ml\ AgNO_3 \left(\frac{1\ liter\ AgNO_3}{1000\ ml\ AgNO_3}\right)\left(\frac{0.0282\ mole\ Ag}{1\ liter\ AgNO_3}\right)\left(\frac{1\ mole\ Cl^-}{1\ mole\ Ag^+}\right)$$

$$= 9.02E\text{-}05\ mole\ Cl^-$$

$$9.02E\text{-}05\ Cl^- \left(\frac{35.45\ g\ Cl^-}{1\ mole\ Cl^-}\right)\left(\frac{}{0.08\ g\ leaf\ tissue}\right) X\ 100$$

$$= 4.00\ \%\ Chloride$$

Appendix D

Electrical Conductivity
(electrolyte leakage)

Preliminary electrical conductivity analysis:

Table D1. Preliminary ion leakage in leaf tissue of soil grown bean plants treated with -0.25 MPa NaCl for 9 days beginning 36 DAP.

NaCl concentration (MPa)	percent ion leakage
-0.25 MPa	20.55 a
0.0 MPa	14.21 b

CALCULATION-PERCENT MEMBRANE LEAKAGE:

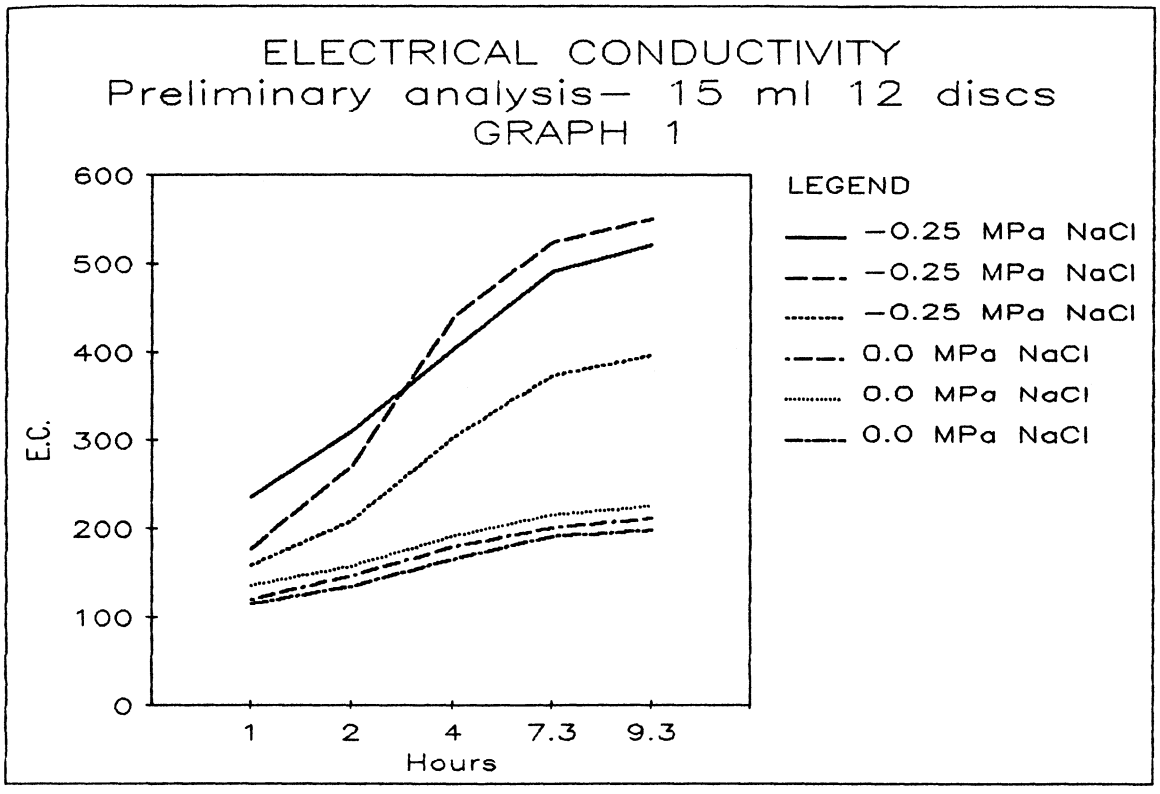
The initial conductivity reading divided by final

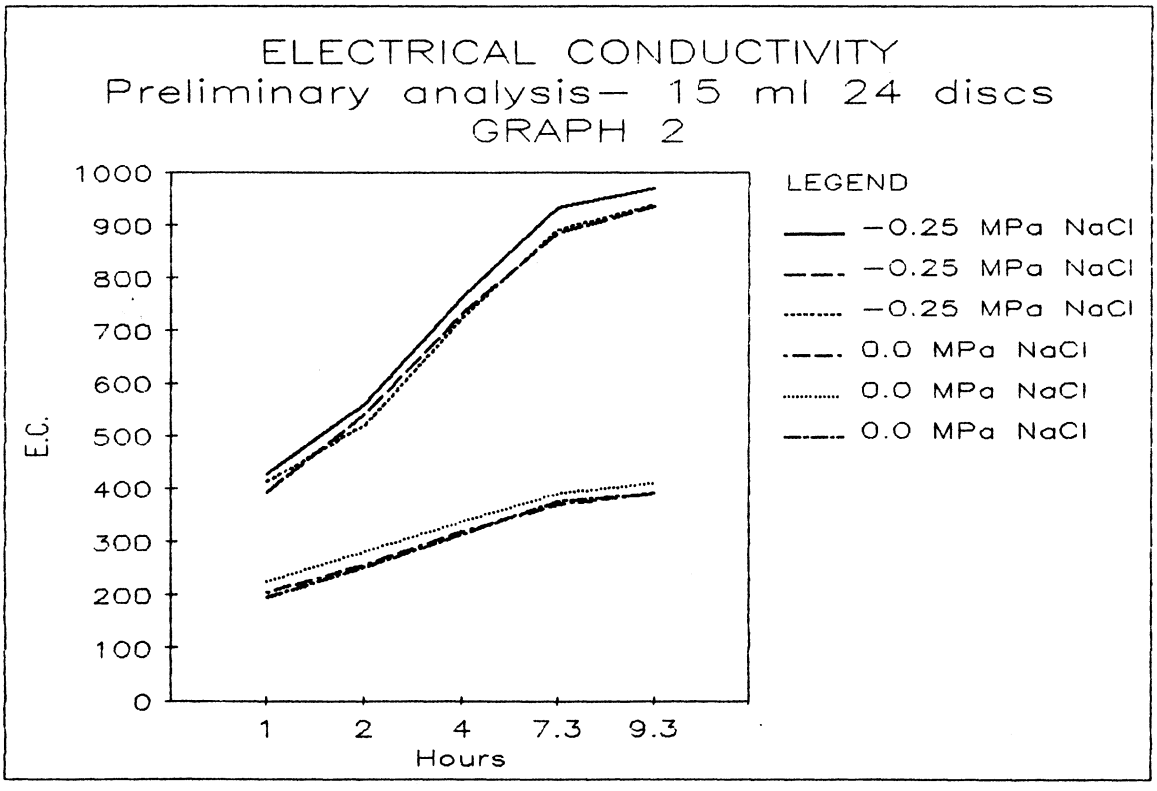
conductivity multiplied by 100.

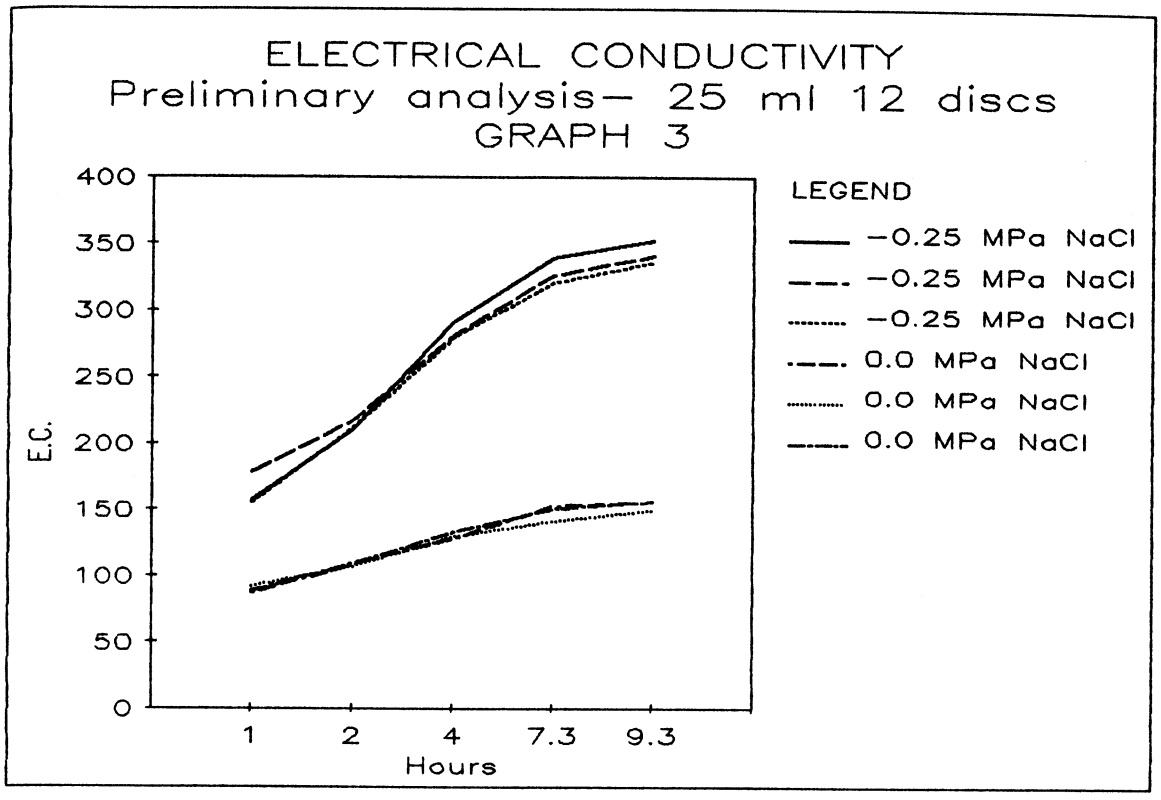
Sample membrane leakage calculation (plant no. 1 upper leaf):

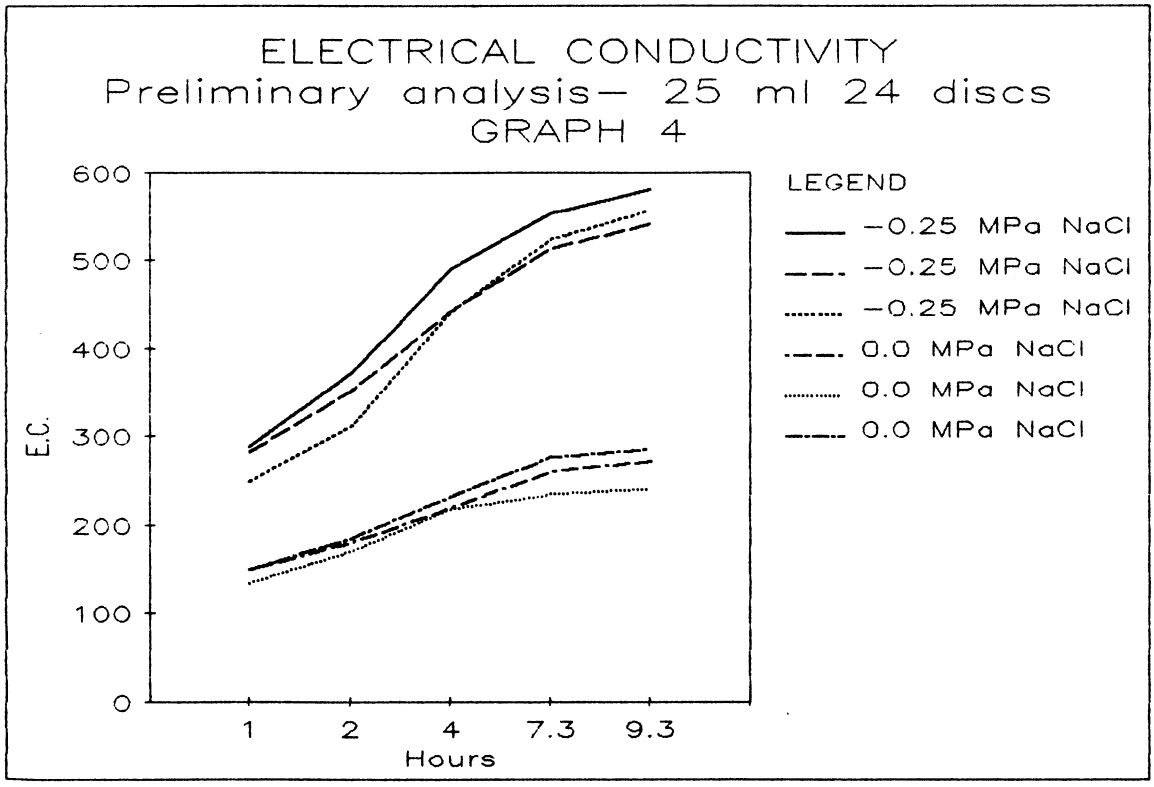
292/830 X 100 = **35.2 percent leakage**

Preliminary ion leakage was measured in leaf tissue of soil grown navy and tepary plants treated with -0.25 MPa NaCl for 9 days beginning 36 DAP. Ion leakage was analyzed for salt treated and control plants. 7 mm leaf discs (12 or 24 per tube) were placed in deionized water (15 ml and 25 ml portions) and electrical conductivity (E.C.) was read for each sample over a period of time (see graphs 1-4 appendix D). Once eqilibrated, a plateau was reached and the E.C. reading at this time represented the "initial" E.C. Preliminary ion leakage analysis was measured as a gauge to determine number of leaf discs and volume of solution to use. Sample size was determined by closeness of data within treatments (see graph 3). Although data for 15 ml and 25 ml portions were similarly close, it was easier to work with 25 ml portions.


ELECTRICAL CONDUCTIVITY
Preliminary analysis— 15 ml 12 discs
GRAPH 1
600
500
400
300
200
100
0
E.C.
1
2
4
7.3
9.3
Hours
LEGEND
—0.25 MPa NaCl
—0.25 MPa NaCl
—0.25 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl


ELECTRICAL CONDUCTIVITY
Preliminary analysis— 15 ml 24 discs
GRAPH 2
1000
900
800
700
600
500
400
300
200
100
0
E.C.
1
2
4
7.3
9.3
Hours
LEGEND
—0.25 MPa NaCl
—0.25 MPa NaCl
—0.25 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl


ELECTRICAL CONDUCTIVITY
Preliminary analysis— 25 ml 12 discs
GRAPH 3
400
350
300
250
200
150
100
50
0
E.C.
1
2
4
7.3
9.3
Hours
LEGEND
—0.25 MPa NaCl
—0.25 MPa NaCl
—0.25 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl


ELECTRICAL CONDUCTIVITY
Preliminary analysis— 25 ml 24 discs
GRAPH 4
600
500
400
300
200
100
0
E.C.
1
2
4
7.3
9.3
Hours
LEGEND
—0.25 MPa NaCl
—0.25 MPa NaCl
—0.25 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl
0.0 MPa NaCl

Appendix E

ANOVA TABLES

Table E1. Three way ANOVA completely randomized-percent sodium

SOURCE	SS	df	MS	F
Main Effects				
part	4.38E-05	3	1.46E-05	8.92***
var	3.08E-06	1	3.08E-06	1.88ns
salt	6.80E-05	1	6.80E-05	41.57***
Interaction				
part x var	1.65E-05	3	5.51E-06	3.37*
part x salt	2.33E-06	3	7.76E-07	0.47ns
var x salt	6.19E-07	1	6.19E-07	0.38ns
part x var x salt	4.02E-06	3	1.34E-06	0.82ns
Error	7.85E-05	48	1.64E-06	

Table E2. Two way ANOVA-percent sodium tepary

SOURCE	SS	df	MS	F
Main Effects				
part	1.30E-05	3	4.35E-06	2.20ns
salt	2.78E-05	1	2.78E-05	14.05***
Interaction				
part x salt	4.69E-06	3	1.56E-06	0.79ns
Error	4.75E-05	24	1.98E-06	

Table E3. Two way ANOVA-percent sodium navy

SOURCE	SS	df	MS	F
Main Effects				
part	4.73E-05	3	1.58E-05	12.20***
salt	4.08E-05	1	4.08E-05	31.60***
Interaction				
part x salt	1.65E-06	3	5.51E-07	0.43ns
Error	3.10E-05	24	1.29E-06	

Table E4. Two way ANOVA-percent sodium young leaves

SOURCE	SS	df	MS	F
Main Effects				
var	2.99E-06	1	2.99E-06	0.84ns
salt	2.25E-05	1	2.25E-05	6.33*
Interaction				
var x salt	8.84E-07	1	8.84E-07	0.25ns
Error	4.27E-05	12	3.56E-06	

Table E5. Two way ANOVA-percent sodium middle leaves

SOURCE	SS	df	MS	F
Main Effects				
var	1.22E-07	1	1.22E-07	0.30ns
salt	9.67E-06	1	9.67E-06	23.51***
Interaction				
var x salt	1.09E-07	1	1.09E-07	0.26ns
Error	4.94E-06	12	4.11E-07	

Table E6. Two way ANOVA-percent sodium old leaves

SOURCE	SS	df	MS	F
Main Effects				
var	8.85E-07	1	8.85E-07	1.27ns
salt	2.47E-05	1	2.47E-05	35.26***
Interaction				
var x salt	8.94E-08	1	8.94E-08	0.13ns
Error	8.39E-06	12	6.99E-07	

Table E7. Two way ANOVA-percent sodium roots

SOURCE	SS	df	MS	F
Main Effects				
var	1.56E-05	1	1.56E-05	8.32*
salt	1.35E-05	1	1.35E-05	7.18*
Interaction				
var x salt	3.55E-06	1	3.55E-06	1.90ns
Error	2.25E-05	12	1.87E-06	

Table E8. Three way ANOVA completley randomized-percent chloride

SOURCE	SS	df	MS	F
Main Effects				
part	0.60	3	0.19	0.27ns
var	0.04	1	0.04	0.06ns
salt	63.32	1	63.32	85.94***
Interaction				
part x var	3.19	3	1.06	1.44ns
part x salt	0.67	3	0.22	0.30ns
var x salt	0.00017	1	0.00017	2.25ns
part x var x salt	3.23	3	1.08	1.46ns
Error	35.36E-05	48	0.74	

Table E9. Three way ANOVA completley randomized-percent ion leakage

SOURCE	SS	df	MS	F
Main Effects				
part	3141.7	1	3141.7	12.19**
var	190.2	1	190.2	0.74ns
salt	583.9	1	583.9	2.27ns
Interaction				
part x var	145.3	1	145.3	0.56ns
part x salt	18.2	1	18.2	0.07ns
var x salt	706.8	1	706.8	2.74ns
part x var x salt	99.2	1	99.2	0.38ns
Error	6183.6	24	257.6	

Appendix F

NONLUMPED MEANS

Table F1. Percent sodium nonlumped means for tepary and navy with three leaf ages and in roots of control and salt treated plants.

	Tepary	
Plant Part	0.00 MPa	-0.25 MPa
young leaf	1.50E-03 ± 5.23E-04[z]	0.0180 ± 0.0290
middle leaf	5.83E-04 ± 3.92E-04	0.0017 ± 0.0011
old leaf	9.78E-04 ± 7.41E-04	0.0029 ± 0.0012
root	2.30E-03 ± 2.88E-04	0.0032 ± 0.0021

	Navy	
Plant Part	0.00 MPa	-0.25 MPa
young leaf	1.1E-03 ± 8.47E-04	0.0030 ± 0.0021
middle leaf	7.3E-04 ± 1.51E-04	0.0024 ± 0.0011
old leaf	8.7E-04 ± 3.92E-04	0.0030 ± 0.0019
root	3.3E-03 ± 5.27E-04	0.0061 ± 0.0017

[z] means ± standard deviations

Table F2. Percent chloride nonlumped means for tepary and navy with three leaf ages and in roots of control and salt treated plants.

	Tepary	
Plant Part	0.00 MPa	-0.25 MPa
young leaf	0.084 ± 0.048[z]	1.70 ± 0.40
middle leaf	0.081 ± 0.051	2.22 ± 0.42
old leaf	0.160 ± 0.084	1.90 ± 0.55
roots	0.061 ± 0.048	2.54 ± 0.98

	Navy	
Plant Part	0.00 MPa	-0.25 MPa
young leaf	0.05 ± 0.034	3.01 ± 2.74
middle leaf	0.06 ± 0.037	2.03 ± 0.88
old leaf	0.04 ± 0.004	1.44 ± 1.49
roots	0.04 ± 0.022	1.33 ± 0.60

[z] means ± standard deviations

Table F3. Percent ion leakage nonlumped means for tepary and navy with two leaf ages of control and salt treated plants.

	Tepary	
Plant Part	0.00 MPa	-0.25 MPa
young leaf	32.98 ± 4.83[z]	52.94 ± 17.72
old leaf	19.44 ± 2.37	35.29 ± 27.56

	Navy	
Plant Part	0.00 MPa	-0.25 MPa
young leaf	45.29 ± 13.25	39.41 ± 27.21
old leaf	16.18 ± 3.27	20.36 ± 5.96

[z] means ± standard deviations

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